

## Protocol

### Transfection for 3'UTR luciferase [p12]

#### 1. CELLS

Plate COS-7 cells in p12 well plate to be at 80% confluence the day of the transfection.

#### 2. STOCK mimics

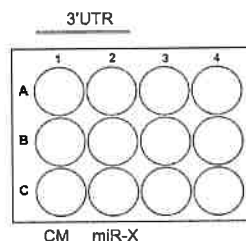
- a. Dissolve stock in 1x siRNA buffer solution (Dharmacon) for a 20  $\mu$ M final concentration of the stock:

5nmol=> Add 250  $\mu$ l

- b. Aliquot and store at -20°C.

#### 3. CALCULATIONS

Each condition will be done in triplicate



##### A. PREPARE MIX 1 [MIMICS]:

- OptiMEM: 50  $\mu$ l per well (X3 wells= 150  $\mu$ l)
- Mimic: 1  $\mu$ l (20nM) per well (X3 wells= 3  $\mu$ l)
- Incubate 5 min at RT

##### B. PREPARE MIX 2 [3'UTR]:

- OptiMEM: 50  $\mu$ l per well (X6 wells= 300  $\mu$ l)
- 3' UTR: Add 1  $\mu$ g of plasmid per well.
- Incubate 5 min at RT.

##### C. PREPARE MIX 3 [LIPO2000]:

- OptiMEM: 50  $\mu$ l per well (X6 wells= 300  $\mu$ l)
- LIPO2000: 2  $\mu$ l per well (X6 wells= 12  $\mu$ l)
- Incubate 5 min at RT.

##### D. PREPARE MIX 4:

- Add 50  $\mu$ l MIX 2 per well (X6 wells= 150  $\mu$ l) in each tube of A.
- Add 50  $\mu$ l MIX 3 per well (X6 wells= 150  $\mu$ l) in each tube of A
- Incubate 30 min at RT.

## Protocol

### E. PREPARE CELLS

- Wash the cells twice in steril PBS 1X .
- Add 300  $\mu$ l of OptiMEM.

### F. ADD MIX 4

- Add 150  $\mu$ l of MIX 4 (final) to its correspondent well. Final vol= 450 $\mu$ l.

### G. ADD 2X MEDIA

- After 8 after transfection add 450  $\mu$ l DMEM 2X (20% of FBS). Final vol= 900 $\mu$ l.

### H. COLLECT CELL

- 24 hrs after transfection.